Supplementary Information for

Optimizing Tenogenic Differentiation of Equine Adipose-Derived Mesenchymal Stem Cells (eq-ASC) Using TGFB3 Along with BMP Antagonists

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Fig.S1: Concentration-dependent effect of GREM2 on tenogenic differentiation. A. eq-ASCs treated with 100 and 200 ng/ml GREM2 showed a little increase in expression of SCX in response to 200 ng/ml at day 3 while decreased compared to control group (1.3 vs. 2.8). B. Morphological changes and qRT-PCR analysis for tenogenic-related genes (SCX, MKX and COL1A1), and C. Osteogenic-related genes (CTNNB, RUNX2, BMPR2) of eq-ASCs in response to 200 ng/ml GREM2 on three-time points are shown (day 3, 7 and 10) (scale bar: 200 μm). Data were normalized to GAPDH and presented as mean ± SD.
*; Present significant changes vs. day 0 untreated cells (dashed line), *P<0.05 and qRT-PCR; Real-time polymerase chain reaction.
Table S1: Culture medium composition for induction of tenogenesis in eq-ASCs

<table>
<thead>
<tr>
<th>Medium</th>
<th>Description</th>
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<tbody>
<tr>
<td>Ctrl</td>
<td>Basic medium: DMEM-high glucose, 10% FBS, 1% P/S</td>
</tr>
<tr>
<td>T</td>
<td>Basic medium+TGF-β3 (2.5 ng/ml)</td>
</tr>
<tr>
<td>T/G</td>
<td>Basic medium+TGF-β3 (2.5 ng/ml)+GREM2 (200 ng/ml)</td>
</tr>
<tr>
<td>T/G/S</td>
<td>Basic medium+TGF-β3 (2.5 ng/ml)+GREM2 (200 ng/ml)+SOST (1250 ng/ml)</td>
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</tbody>
</table>

Ctrl; Control, T; Treatment with TGF-β3 (2.5 ng/ml) for one day, T/G; Pretreatment with TGF-β3 (2.5 ng/ml) followed by GREM2 (200 ng), and T/G/S; Pretreatment with TGF-β3 (2.5 ng/ml) followed by GREM2 (200 ng/ml) and SOST (1250 ng/ml).

Table S2: Primer sequences which were used for real-time polymerase chain reaction

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession Number</th>
<th>Primer sequence (5’-3’)</th>
<th>Amplicon length (bp)</th>
</tr>
</thead>
</table>
| GAPDH  | NM_001163856.1   | F: GTGCTGAATATGGTGTGGAGT  
R: AGAAGGAGCAGAGATGATGAC | 104 |
| SCX    | NM_001105150.1   | F: GACGCCCGCAGCCAACA  
R: CATCCGCTCTAATCCGAATC | 103 |
| MKX    | XM_014737017.1   | F: AAAATCCCCGTTCACCATCCTG  
R: TTTGCCCTTGTCTTTCCCATCAT | 196 |
| COL1A1 | XM_023652710.1   | F: CGGGTTTGGAGGAAGTCGG  
R: ACGAGGTAGTCTTTCCAGCAAC | 140 |
| TNMD   | NM_001081822.1   | F: TTTTCACTCACCAATCCAGCA  
R: AATAACCTCTCCTATCCAGCA | 179 |
| SOX9   | XM_014736619.1   | F: ATTCAGAACACAGAATTCG  
R: ACGGTTTTCAACTCCATCCT | 157 |
| CTNNB1 | NM_001122762.1   | F: ACTGTCTCTTCTTGCTGGTGAC  
R: AGTGGGATGGTGATGTAGGA | 163 |
| BMPR2  | XM_014732300.1   | F: GACTCTGCTACTCCTAACATCC  
R: TCCTACGGGTGAATTAAAGC | 158 |
| RUNX2  | XM_005603968.2   | F: ACGCATCTACACTATGTCAG  
R: GGGAAGACAGACTAAAGGAC | 133 |